DNA CAPILLARY ELECTROPHORESIS SETUP FOR A COMBINED GLOBALFILER AND YFILER PLATE USING THE EPMOTION

A. SCOPE

A.1 The capillary electrophoresis setup process consists of multiple transfers of liquids containing either reagents or DNA from one place to another. By utilizing the epMotion 5075, a liquid handling robot, the incidence of human error and/or the introduction of contamination in this process can be minimized. Furthermore, automation of the capillary electrophoresis setup process allows for analysts to complete other tasks while these steps are being performed.

B. QUALITY CONTROL

B.1 A lab coat and protective gloves must be worn when performing this procedure to prevent contamination.

B.2 See DOC ID 1835 to determine reagent expiration dates.

B.3 Hi-Di Formamide: To prevent repeated thawing and re-freezing of formamide, aliquot formamide into approximately 500 and 1000 µL volumes after initial thawing of the 25 mL bottle. Appropriately discard any unused aliquot of thawed formamide.

C. SAFETY

C.1 A lab coat and protective gloves must be worn when performing this procedure. Eye protection (e.g. safety glasses or a face shield) must also be worn when performing any parts of this procedure outside of the epMotion hood.

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

C.3 Hi-Di formamide: exposure causes eye, skin, and respiratory tract irritation. It is also a possible developmental and birth defect hazard.

D. REAGENTS, STANDARDS AND CONTROLS

D.1 GlobalFiler allelic ladder

D.2 YFiler allelic ladder

D.3 GS600 LIZ internal size standard

D.4 GS500 LIZ internal size standard

D.5 Hi-Di formamide

D.6 3130 Performance Optimized Polymer (POP-4 polymer)

D.7 AB 3130 Genetic Analyzer 10X Buffer w/EDTA.

D.7.1 To make a 1X working buffer:

Add 25 mL of 10X Buffer to 225 mL of DI H_2O to make 250 mL of working buffer or add 100 mL of 10X Buffer (4 bottles) to 900 mL DI H_2O to make 1000 mL of working buffer.

D.8 70% ethanol (decontamination of the epMotion 5075)

D.9 DNA-Exitus Plus (decontamination of the epMotion 5075)

D.10 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (decontamination of the epMotion 5075 waste container)

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 epMotion 5075 (instrument, computer, and appropriate software)

E.1.2 epMotion dispensing tools

E.1.3 epMotion labware (thermoblocks, reservoir rack, and module racks)

E.1.4 AB 3130 Genetic Analyzer (instrument, computer, and appropriate software)

E.1.5 AB 36 cm capillary array

E.1.6 AB 3130 Genetic Analyzer sample septa and plates

E.1.7 Thermal cycler

E.1.8 Pipettes

E.1.9 Vortexer

E.1.10 Frozen plate block

E.1.11 Decapper

E.1.12 96-well plate retainer and base

E.1.13 96-well plate centrifuge

E.2 Supplies

E.2.1 3130 Genetic Analyzer buffer vials/reservoirs/reservoir septa

E.2.2 Pipette tips

E.2.3 epMotion supplies (5 mL tubes, epT.I.P.S. Motion 1-50 µL tips, epT.I.P.S. Motion 20-300 µL tips)

E.2.4 Strip caps

E.2.5 Parafilm

E.2.6 50 mL conical tubes

E.2.7 Scalpel

E.2.8 Permanent marker

F. PROCEDURES

F.1 Create a 3130 plate map using the following highlighted locations for GS600 LIZ/formamide blanks, GlobalFiler allelic ladders, and YFiler allelic ladders. The locations of the amplified samples will vary depending on the number of samples.

***Note**: The 3130 plate map needs to be designed in such a way that air will not be injected onto the multi-capillary array and so that an entire column of wells is used, e.g. if 17 GlobalFiler amplified samples are to be pipetted into wells A2-A4, GS600 LIZ/formamide should still be dispensed into wells B4-H4 as shown on the plate map example. In addition, with this method, YFiler amplified samples must be setup starting with the first unused "A" well, e.g. if 11 YFiler samples are to be pipetted onto the aforementioned plate, the first sample should be pipetted into well A5 and GS500 LIZ/formamide should be dispensed into well D6 as shown on the plate map example.*

	1	2	3	4	5	6
A	*600-LIZ*	GF-Sample 1	GF-Sample 9	GF-Sample 17	Y-Sample 1	Y-Sample 9
B	*600-LIZ*	GF-Sample 2	GF-Sample 10	600-LIZ	Y-Sample 2	Y-Sample 10
C	*600-LIZ*	GF-Sample 3	GF-Sample 11	600-LIZ	Y-Sample 3	Y-Sample 11
D	*600-LIZ*	GF-Sample 4	GF-Sample 12	600-LIZ	Y-Sample 4	500-LIZ
E	*GF-LADDER*	GF-Sample 5	GF-Sample 13	600-LIZ	Y-Sample 5	
F	*GF-LADDER*	GF-Sample 6	GF-Sample 14	600-LIZ	Y-Sample 6	
G	*Y-LADDER*	GF-Sample 7	GF-Sample 15	600-LIZ	Y-Sample 7	
H	*Y-LADDER*	GF-Sample 8	GF-Sample 16	600-LIZ	Y-Sample 8	

GF = GlobalFiler
Y = YFiler

F.2 Import or create a plate document for the 3130 Genetic Analyzer as described in sections F.3 and F.4 of DOC ID 1766. The plate document does not have to be prepared at this step; it can be prepared at any time.

F.3 Combine the necessary amount of formamide and GS600 LIZ in an Eppendorf 5 mL tube as follows:

(Number of samples + 2) x 19.2 µL formamide

(Number of samples + 2) x 0.8 µL GS600 LIZ

***Note**: It is recommended that enough volume for additional samples be included in the calculation to account for volume lost in pipetting.*

F.4 Briefly vortex the mixture.

F.5 Combine the necessary amount of formamide and GS500 LIZ in an Eppendorf 5 mL tube as follows:

(Number of samples + 2) x 24.5 µL formamide

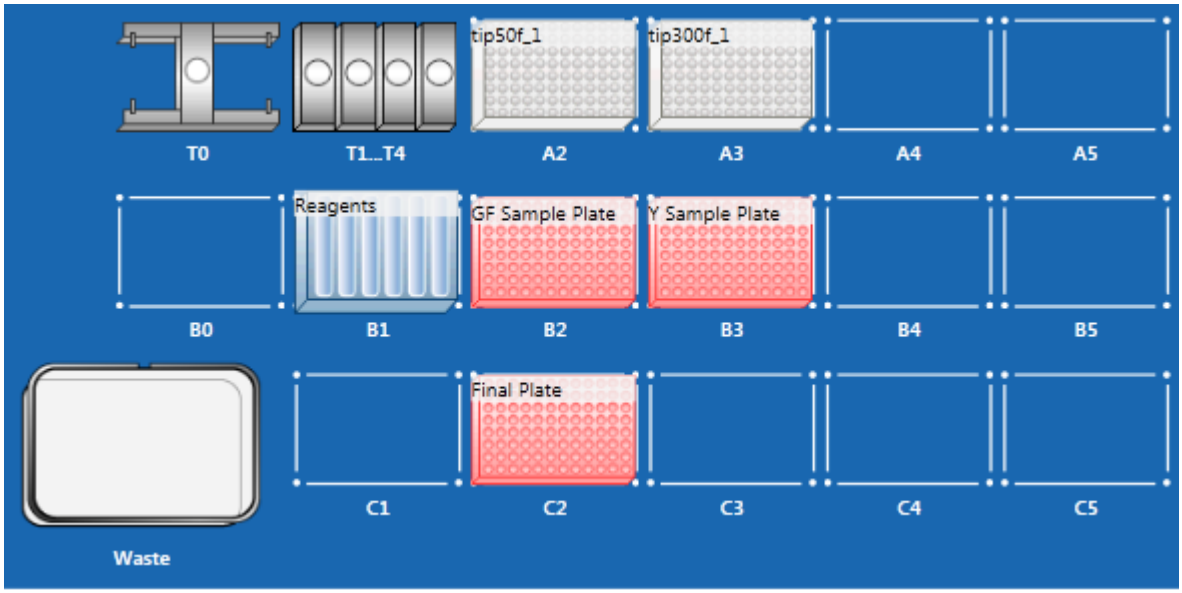
(Number of samples + 2) x 0.5 µL GS500 LIZ

***Note**: It is recommended that enough volume for additional samples be included in the calculation to account for volume lost in pipetting.*

F.6 Briefly vortex the mixture.

F.7 Prepare the epMotion worktable with tips, dispensing tools, the GlobalFiler amplification plate (with strip caps removed), the YFiler amplification plate (with strip caps removed) and an empty 96-well plate as shown below; for details on this preparation please refer to the "Capillary Electrophoresis Setup for a Combined PowerPlex16 HS and YFiler Plate Using the epMotion 5075" procedure.

Note: The amplification plates must be centrifuged briefly before placing them on the robot worktable; these plates may also be added to the worktable after preparing the reagent reservoir rack, i.e. after Step F.8.3.



F.8 Prepare the reagent reservoir rack as follows:

F.8.1 Place an open 5 mL tube containing the GS600 LIZ/formamide master mix in position 4C of the reservoir rack with the tube lid in the lid holder.

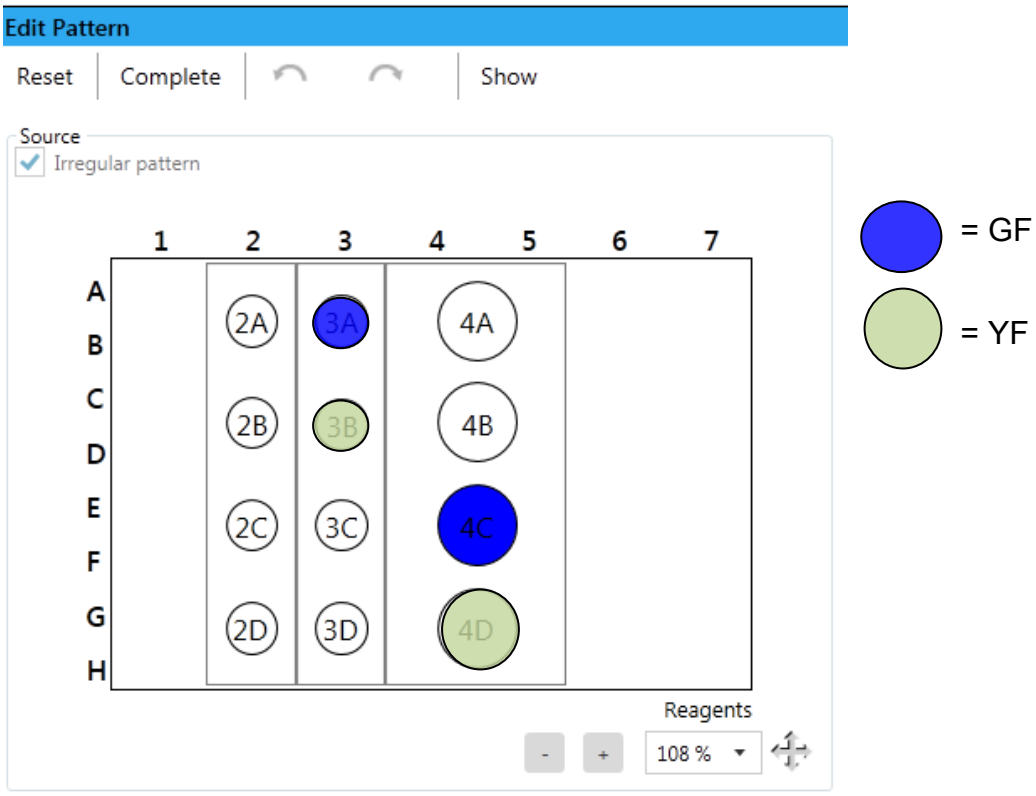
F.8.2 Place an open 5 mL tube containing the GS500 LIZ/formamide master mix in position 4D of the reservoir rack with the tube lid in the lid holder.

Place an open tube containing GlobalFiler allelic ladder in position 3A of the reservoir rack.

***Note**: A minimum volume of 11 µL of GlobalFiler allelic ladder is required for this epMotion protocol.*

F.8.3 Place an open tube containing YFiler allelic ladder in position 3B of the reservoir rack.

***Note**: A minimum volume of 10 µL of YFiler allelic ladder is required for this epMotion protocol.*



F.9 Close the front hood of the epMotion.

F.10 Open the Eppendorf epBlue software.

F.11 Select **Application Editor** from the main menu, **DNA**; **3130 plate set-up**; **Global-YFiler_Setup.dws** method in the DNA folder.

F.12 The Global-YFiler_Setup method must be modified with every combined plate setup performed; this modification is necessary to account for the variable nature of the number of GlobalFiler and YFiler amplified samples.

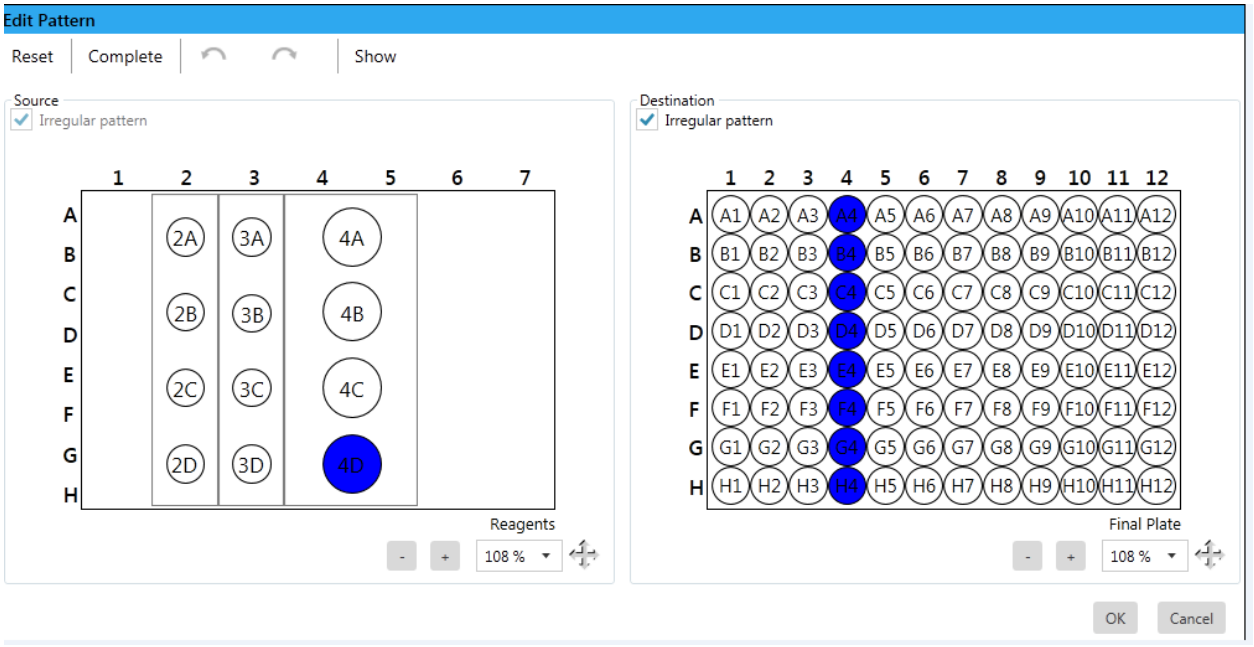
F.12.1 The Global-YFiler_Setup method is "read only"; therefore, click the **Save As icon** and name the application with your 3130 run name so that the following method modifications can be made.

F.12.2 Click on the **Switch to Procedure** button and then click on **Step 7**. Using your 3130 plate map as a guide, enter a number (up to 88) in the **Number of Samples** location; this will be the number of wells on the 3130 plate (final plate) that the GS600 LIZ/formamide mixture will be added to for GlobalFiler amplified samples (including any blanks used to fill a sample column on the plate). This number should not include the wells that will contain GS600 LIZ/formamide blanks and GlobalFiler allelic ladders in the first column of the plate. In the previous plate map example this number would be 24. Click the **Save Icon**.

F.12.3 (OPTIONAL) Click on **Step 8.** Click on **Pattern…** and then click on to check that the GS600 LIZ/formamide mixture will be dispensed into the desired wells.

F.12.4 Click on **Step 9**. Using your 3130 plate map as a guide, enter a number (up to 88) in the **Number of Samples** location; this will be the number of wells on the 3130 plate (final plate) that the GS500 LIZ/formamide mixture will be added to for YFiler amplified samples (including any blanks used to fill a sample column on the plate). This number should not include the wells that will contain YFiler allelic ladders in the first column of the plate. In the previous plate map example this number would be 12. Click the **Save Icon**.

F.12.5 Click on **Step 10 - Reagent Transfer** and then click on **Pattern**. For both "Source" and "Destination" check the Irregular pattern box. Highlight by clicking the 4D source and then highlight by clicking each well the GS500 LIZ/formamide mixture will be added to as shown below. Click **OK**. Click the **Save Icon**.



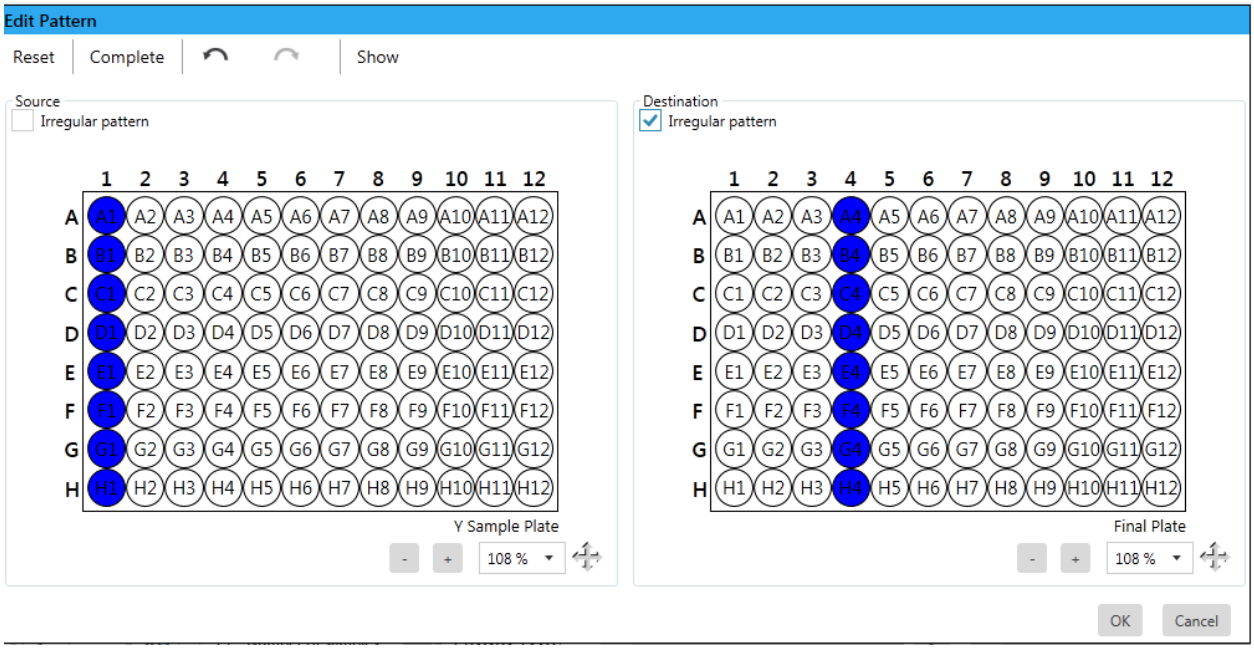
F.12.6 Click on **Step 17 - Number of Samples** in the Procedure tab. Using your 3130 plate map as a guide, enter a number in the **Number of Samples** location; this will be the number of GlobalFiler amplified samples to be added to the 3130 plate (including any blanks used to fill a sample column on the plate). This number should be the same as the number that was entered at Step 7. Click the **Save Icon**.

F.12.7 (OPTIONAL) Click on **Step 18 - Sample Transfer** and then click on **Pattern…** Click on **Show** to check that the GlobalFiler amplified samples will be dispensed into the desired wells.

Note: For this combined method, the GlobalFiler amplified samples should always be added to the A2 column first, followed by the A3 column, etc.

F.12.8 Click on **Step 19 - Number of Samples** in the Procedure tab. Using your 3130 plate map as a guide, enter a number in the **Number of Samples** location; this will be the number of YFiler amplified samples to be added to the 3130 plate (including any blanks used to fill a sample column on the plate). This number should be the same as the number that was entered at Step 9. Click the **Save Icon**.

F.12.9 Click on **Step 20 - Sample Transfer** and then click **Pattern**. For the "Destination" check the Irregular pattern box. Highlight by clicking on the A1 well of the "Source plate" labeled Y Sample Plate. Then highlight by clicking the first well of the column these samples should be added to. The program will highlight the rest. Continue column by column until all samples are highlighted. Click **OK**.

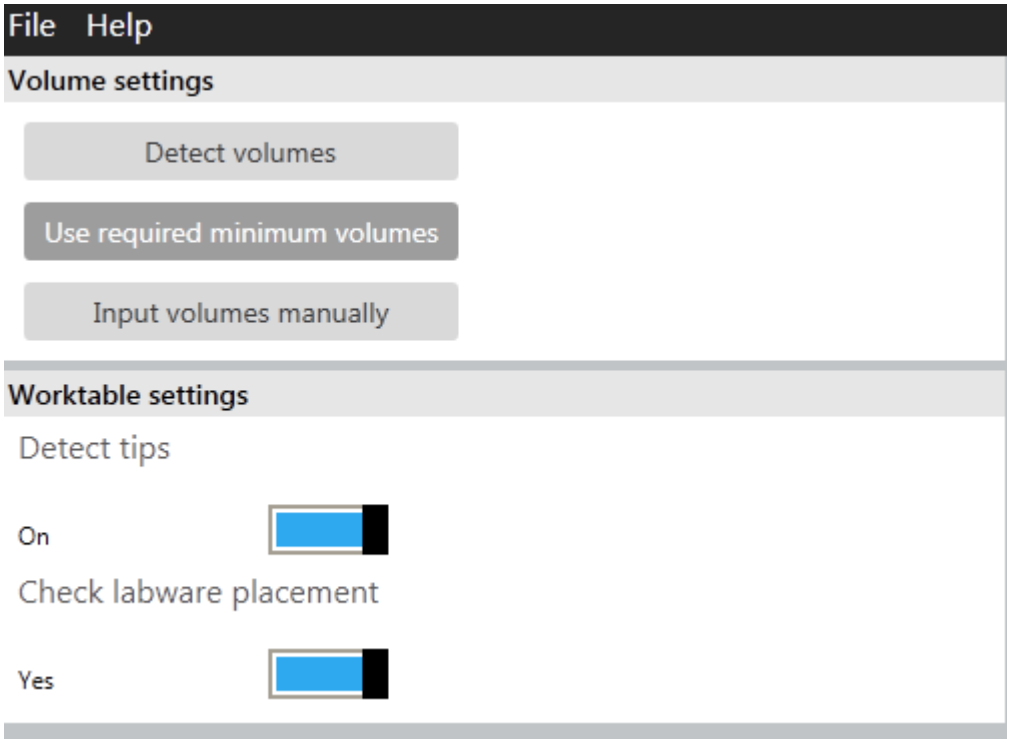


F.12.10 **Click the Save Icon** to save the modified method.

F.13 Click the gray triangle on the toolbar to start the modified method.

F.14 Ensure that compatible devices is selected and that device 5075YN901513 is highlighted, click **Next.**

F.15 Ensure that **Use required minimum volumes, Detect tips, and Check labware placement** are selected on the following screen



***Note**: The level sensor will check the type and quantity of tips present on the worktable. However, this sensor does not determine if there are enough tips for the entire run; the method will continue until all tips have been used and then the process will stop and the software will prompt you to insert more tips.*

Only four 20-300 µL tips are needed to dispense the GS600 LIZ/formamide and GS500 LIZ*/formamide but four 1-50 µL tips are needed for the allelic ladders and a variable number of 1-50 µL tips are required for the amplified samples, i.e. if 40 total GlobalFiler and YFiler amplified samples will be setup, 40 tips (in five complete columns of the tip box) are needed for multi-channel dispensing.* The robot can use two types of tip versions. If using the older version the operator **MUST VERIFY that the box had sufficient tips for pipetting and has no tips out of place from the first tip position.**

F.16 Click, **Run** and the application will start and the display will switch to the Control tab. The progress and current status of the method will be displayed. A message will appear when the method has been completed.

F.17 To stop the method before it is complete, click the **Pause** icon (red square) in the Control tab or lift the front hood. Then click the **Cancel** icon to abort the method; the front hood must be down to abort the method. Alternatively, after stopping the method, click the **Run** icon to continue the method.

F.18 After completion of the method, click **OK** and select file **Exit to Home Screen**, click on **Log Viewer** select your log file by finding the file with the appropriate date and time. Select the **Print Icon** and click **PDF** to save the file as a PDF document. This file should be saved in the appropriate analyst's casework folder on the I drive. A run completed without errors will have "Program ended successfully" on the last line of the log file.

F.19 Open the front hood and remove the 3130 plate and place it into a plate base. Cover the plate with a rubber septa.

F.20 Centrifuge the plate briefly.

F.21 Heat the samples in a thermal cycler for three minutes at 95°C to denature.

F.22 Snap-cool immediately for a minimum of three minutes in a frozen plate holder.

F.23 Place the plate into the plate base and centrifuge briefly.

F.24 Secure the 3130 plate with a plastic retainer clip.

F.25 Place the tray onto the 3130 autosampler with position A1 at the back right.

F.26 Link the 3130 plate to a run by clicking on the yellow plate diagram. Select the green arrow from the Run Scheduler window to start the run; see sections F.5 of DOC ID 1766 for additional information.

Note: When the run is complete, a copy of the raw data should be saved in the appropriate analyst's casework folder on the I drive. The analyst should maintain case folders in monthly files. The data from the first analysis of any convicted offender or arrestee sample should be stored under K:\Division\DNA\CODIS\Analysis.

F.27 Prepare the epMotion worktable for the next user:

F.27.1 Add strip caps to the GlobalFiler and YFiler amplification plates and store them in a refrigerator protected from evaporation.

F.27.2 Cap the GlobalFiler and YFiler allelic ladder tubes and remove them from the reservoir rack.

F.27.3 Discard any unused GS600 LIZ/formamide and GS500 LIZ/formamide mixture.

F.27.4 Empty the waste container and decontaminate it with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner.

F.27.5 Wipe down the epMotion deck with 70% ethanol solution or DNA Exitus Plus cleaning solution.

G. INTERPRETATION GUIDELINES

G.1 See DOC ID 12628 (GlobalFiler Interpretation Guidelines) and 1776 (YFiler Interpretation Guidelines).

H. REFERENCES

H.1 Eppendorf epMotion 5075 with Integrated PC and epBlue Operating Manual, 2008.

H.2 epMotion Validation Binder 6, Global-YFiler_Setup.dws method.